Aquatic Invasions (2011) Volume 6, Issue 4: 391–403
doi: 10.3391/ai.2011.6.4.04

Proceedings of the 3rd International Invasive Sea Squirt Conference, Woods Hole, USA, 26–28 April 2010

Research Article

Monitoring for invasive tunicates in Nova Scotia, Canada (2006-2009)

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Received: 7 December 2010 / Accepted: 1 June 2011 / Published online: 19 September 2011

Editor's note:
This paper is a contribution to the proceedings of the 3rd International Invasive Sea Squirt Conference held in Woods Hole, Massachusetts, USA, on 26–28 April 2010. The conference provided a venue for the exchange of information on the biogeography, ecology, genetics, impacts, risk assessment and management of invasive tunicates worldwide.

Abstract

The shellfish culture industry in Atlantic Canada has been adversely affected by the presence of non-indigenous, invasive tunicates since the mid-1990's. A Fisheries and Oceans Canada Aquatic Invasive Species (DFO-AIS) monitoring program documented the presence, establishment, and spread of five tunicate species at geo-referenced coastal monitoring stations in Nova Scotian waters from 2006-2009. *Styela clava* (Herdman, 1881) and *Didemnum vexillum* (Kott, 2002) were not found in Nova Scotia during the course of this study, despite their problematic presence in Prince Edward Island and the Gulf of Maine, respectively. *Botryllus schlosseri* (Pallas, 1766) was the most widely distributed species, found at more than 69% of sites monitored in all years. *Ciona intestinalis* (Linnaeus, 1767) was present at about half of the stations in all years, and while its populations were heaviest and most persistent in the Halifax – St. Margaret's Bay, Shelburne – Port La Tour, and Canso – Isle Madame areas, there was evidence of spread on the eastern and Fundy shores, and in Cape Breton. *Botrylloides violaceus* (Oka, 1927), was the least common tunicate encountered, but its distribution increased from 19% of stations in 2006 to 50% of stations in 2009. Tunicates occupied a wide variety of hard substrates (natural and artificial) in waters with 13.0 to 33.2 salinity and at oxygen saturations of 32.5 to 124.8%.

Key words: ascidians, fouling organisms, monitoring network, distribution

Introduction

Non-indigenous ascidians, or tunicates, have become a concern to global marine ecosystems in recent years due to their adverse effects on native species (Lambert 2001; Lutz-Collins et al. 2009) and communities (Blum et al. 2007; Dijkstra et al. 2007). Once established, invasive tunicates may become the dominant members of fouling communities (Lambert and Lambert 1998, 2003). In practical terms, fouling tunicates are regarded as nuisance species, as they attach and grow on artificial surfaces such as pontoons, wharf pilings, buoys, and boat hulls which must be periodically cleaned. Tunicates pose a greater threat to shellfish aquaculture operations as they can attach to and overgrow aquaculture gear and bivalves (Carver et al. 2003; Bullard et al. 2007a), resulting in increased operation and production costs as gear must be cleaned more frequently, and tunicates removed from shellfish during processing. Invasions of non-indigenous tunicates have resulted in serious economic losses to the blue mussel, *Mytilus edulis* (Linnaeus, 1758), aquaculture industry in Atlantic Canada since the 1990's (Boothroyd et al. 2002; Clarke and Therriault 2007; Howes et al. 2007).

Four species of non-native tunicates are now established in Prince Edward Island. The solitary clubbed tunicate, *Styela clava* (Herdmann, 1881), present since 1997 (Locke et al. 2007) has been replaced by the solitary vase tunicate, *Ciona intestinalis* (Linneaus, 1776), present since 2004 (Locke et al. 2009), as the predominant threat to the mussel aquaculture industry (Ramsay et al. 2008). Two colonial species, the golden star tunicate *Botryllus schlosseri* (Pallas, 1766), present since 2001, and the violet tunicate *Botrylloides violaceus* (Oka, 1927) present since 2002 (Locke et al. 2009), have spread to many areas. These colonial species are regarded as the least problematic of the PEI tunicate invaders (Locke et al. 2009), because *S. clava* and *C. intestinalis* are more aggressive competitors (Carver et al. 2006a; Clarke and Therriault 2007) and are the dominant tunicate foulers in terms of biomass on mussel farms (Thompson and MacNair 2004; Lutz-Collins et al. 2009).

In Nova Scotia, *C. intestinalis* is regarded as a cryptogenic species (Carver et al. 2006a) and is thought to have been present in Atlantic Canadian waters since the 1850's (Stimpson 1852). Heavy colonization was first noted on a mussel farm in the Lunenburg-Mahone Bay area in 1997 (Cayer et al. 1999; Carver et al. 2003), and large populations have subsequently been documented in the Lobster Bay area on the southwestern coast, and on Isle Madame, Cape Breton (Clancey and Hinton 2003; Clancey and MacLachlan 2004) (Figures 1 and 2). Of the non-indigenous colonial tunicates, *B. schlosseri* has been observed in Nova Scotia for several decades (Carver et al. 2006b), while *B. violaceus* has been present since 2001 (Carver et al. 2006b). *Styela clava* has not yet been reported in Nova Scotian waters, but there is concern regarding its potential arrival through transfers of shellfish products, or via commercial or recreational boating vectors. A fifth tunicate species, the colonial pancake batter tunicate *Didemnum vexillum* (Kott, 2002), is present nearby on the American side of Georges Bank (Bullard et al. 2007a; Valentine et al. 2007) and in the Gulf of Maine as far north as Eastport, Maine (Martin et al. 2010). This potential new invader could be carried onshore by currents, attached to scallops shells, or gear returning to Nova Scotian waters.

In response to the growing threat posed by aquatic invasive species, specifically non-indigenous tunicates, to native coastal communities, fisheries, and the shellfish aquaculture industry in Canadian waters, Fisheries and Oceans Canada developed an Aquatic Invasive Species (AIS) Monitoring Program in the spring of 2006. Here, we report on the initial four years (2006–2009) of tunicate monitoring in coastal Nova Scotia.

Methods

Monitoring stations

General monitoring stations: 2006–2009

Coastal and inland (Bras D'Or lakes) general monitoring stations (Appendix 1; Figures 1 and 2) were selected based on the presence of, or potential for, known vectors for the introduction or spread of tunicates (Lambert and Lambert 1998; Therriault and Herborg 2007). These included: (1) presence of shellfish or mussel processing facilities, (2) mussel or shellfish aquaculture sites, (3) important commercial ports with international traffic, (4) marinas or yacht clubs with international traffic either from the USA or other countries of origin, or high volumes of regional traffic, (5) commercial fishing harbours, and (6) harbours with fish, shellfish and/or lobster processing (with catches from Atlantic or US waters). Only sublittoral sites were selected, and preference was given to locations with floating docks, ensuring that collectors remained submerged and off bottom at low tide.

Beginning in 2006, partnerships were established between DFO – Maritimes Region, the Nova Scotia Department of Fisheries and Aquaculture, Fishermen's and Scientist's Research Society, Clean Annapolis River Project, Cape Breton University – Project Unidentified Foreign Organisms (PUFO), DFO – Gulf Region, Unama'ki Institute of Natural Resources, Eskasoni Fisheries and Wildlife Commission, and several shellfish growers, so that a maximum number of stations could be established and monitored. Partners were provided with a standard protocol and monitoring collectors, and reported their observations at the end of each year.

Targeted monitoring stations: 2006

In addition to the general monitoring stations selected in 2006, three areas were selected for an increased (targeted) monitoring effort between July and October. These areas were identified in earlier surveys (Clancey and Hinton 2003; Clancy and MacLachlan 2004) as having problematic infestations of *Ciona intestinalis*. Two areas, each covering about 50 km of coastline, were monitored on mainland Nova Scotia, with 9 stations in Area (1): Halifax – St. Margaret's Bay, and 8 stations in Area (2): Sandy Point – Port La Tour (Figure 1). Nine stations (numbers

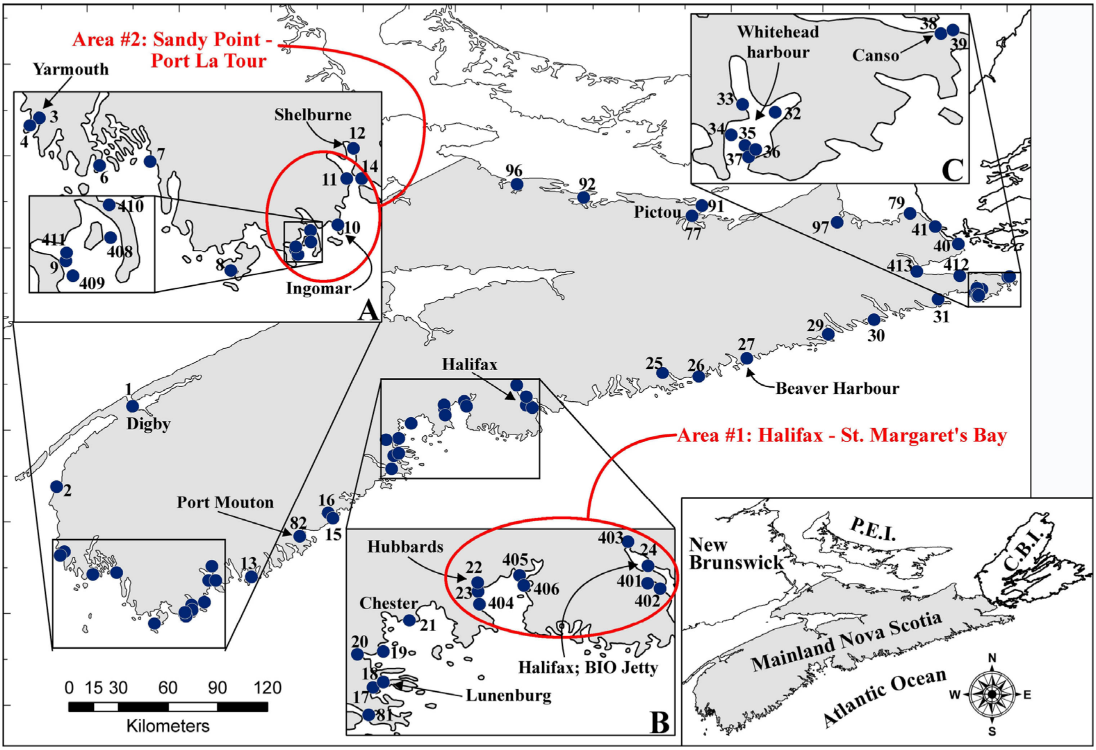

Figure 1. General and targeted monitoring stations in mainland Nova Scotia, 2006-2009. Inset **A** shows stations in the Shelburne to Yarmouth area and Inset **B** shows stations in the Halifax to Lunenburg area. Targeted monitoring areas are circled in red; Area # 1: Halifax to St. Margaret's Bay, and Area #2: Sandy Point to Port La Tour. Station names and descriptions are given in Appendix 1.

38, 40, 42, 43, 46, 47, 412, 413 and 417) within a 50 km radius of Chedabucto Bay were monitored in Area (3): Canso – Isle Madame (Figure 2). Station numbers 44, 45 and 80 in Area 3 were not included, as they were established after the 2006 field season.

Monitoring collectors

Collectors deployed in 2006 consisted of a shading, flowerpot saucer (25 cm diameter) with three attached Petri dishes and six sanded, 10 cm × 10 cm square PVC plates attached to a rope line, which passed through the centre of the saucer (Figure 3A). The distance from the saucer to the bottom plate was approximately 1 m. Collectors were tied to the underside of floating docks, or from wharves, buoys, or lines so that the top of the saucer was at least 1 m below the water surface at low tide. Collectors were hung at the same depth as the mussel lines or oyster cages on shellfish aquaculture sites. Lines were weighted at the bottom to ensure that collectors hung vertically in the water column. From 2007 onward, a modified monitoring collector consisting of a rope line with three sanded, 10 cm × 10 cm square PVC plates spaced 20 – 30 cm apart (Figure 3B) was used. The only exception was PUFO stations monitored in Cape Breton in 2008 (Stns 56 – 73, and 76), where collectors consisted of six PVC plates on the rope line.

Monitoring protocol

Duplicate collectors were deployed in late May – early June and retrieved in mid – late October to assess biofouling from spring to fall, hereafter referred to as a "full deployment". Collectors were also deployed for shorter periods; two collectors in late May – early June for retrieval in mid August (first deployment), and two in mid-August for retrieval in mid – late October

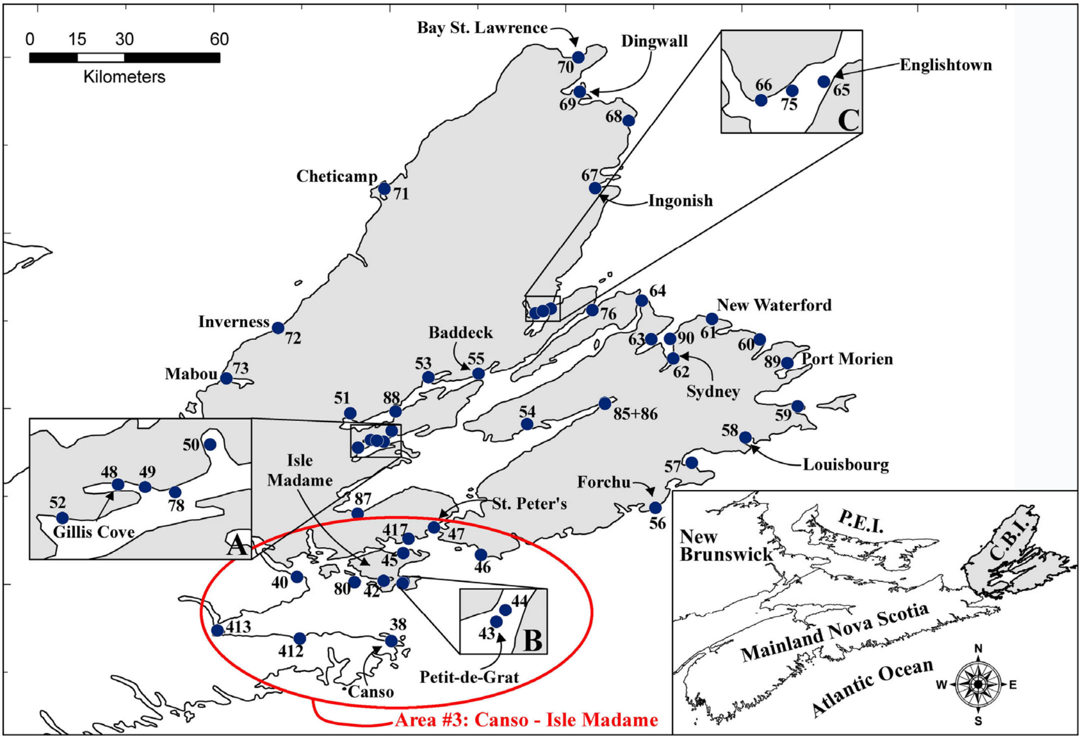

Figure 2. General and targeted monitoring stations in Cape Breton Island (C.B.I.), 2006-2009. Inset **A** shows stations in the Baddeck to Orangedale area, Inset **B** shows stations in the Petit-de-Grat area and Inset **C** shows stations in the Englishtown area. Targeted monitoring Area # 3: Isle Madame to Canso is circled in red. Station names and descriptions are given in Appendix 1.

(second deployment). This protocol ensured that biofouling could still be assessed in the event of heavy fouling on full deployment collectors or if some collectors were lost. First and second deployment collectors also facilitated the detection of species when larvae settled earlier or later in the year. An exception was the 2008 PUFO stations listed above, where only one collector was deployed in each period, and full deployment collectors only were deployed at Stns 48 – 54 and Stn 78 in 2008 (see Appendix 1 for details).

A Garmin GPS Legend or eTrex Unit (Garmin International, Inc., Olathe, Kansas, USA) was used to determine the position of each monitoring station and photographs of deployment structures were taken. Temperature (°C), salinity, conductivity (mS cm^{-1}), and oxygen content (% saturation and mg L^{-1}) were measured using a YSI 85 or YSI Professional Plus meter and probes (2006–2008) or a YSI 6600 V2–4 sonde (2009) (YSI Incorporated, Yellow Springs, Ohio, USA) at each deployment and retrieval. A Hydrolab-Quanta (Hach Hydromet, Loveland, Colorado, USA) instrument was used at Stn 1 (Digby). VEMCO (AMIRIX Systems Inc., Halifax, Nova Scotia, Canada) minilog-T 8k temperature recorders were attached to one of the full season monitoring collectors and deployed at several stations beginning in 2007. A 10-minute visual examination of substrates and structures near the collectors was made at each station to document the presence of tunicates and invasive species including (but not discussed in this paper) *Membranipora membranacea* (Linnaeus, 1767), *Caprella mutica* (Schurin, 1935), *Carcinus maenus* (Linnaeus, 1758), and *Codium fragile* ssp. *fragile* (Suringar) Hariot.

Collectors were removed and usually examined in the field for the presence of tunicates. In 2006, collector plates and Petri dishes were returned to the laboratory, and preserved in 4% formalin for subsequent examination. Beginning in 2008, the collector

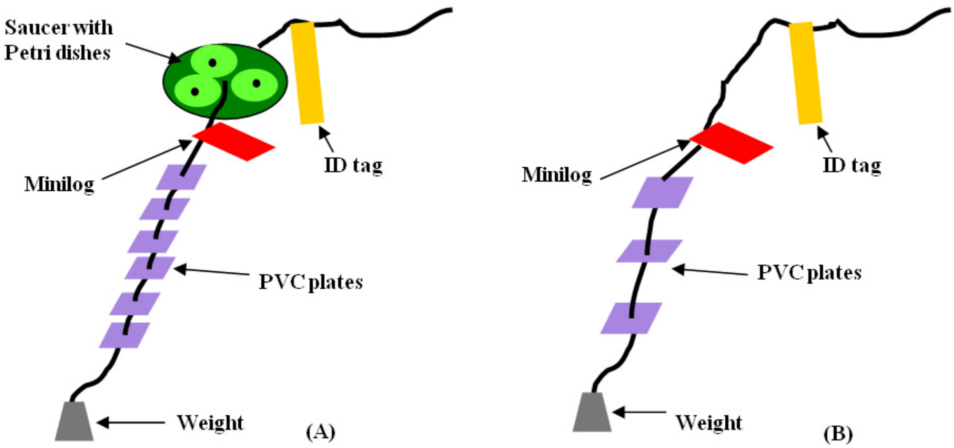

Figure 3. Field monitoring (settlement) collectors used in 2006 (**A**), and 2007 – 2009 (**B**) in Nova Scotia, Canada.

plates were photographed in the field using a Canon Powershot A260 (Canon Canada Inc., Mississauga, Ontario) digital camera. In 2009, plates were photographed in the field or in the laboratory inside a white photographic tent with a mounted camera and a white, labelled background. Digital images were archived for future reference. The presence of tunicates on station substrates or collector surfaces other than the plates (saucer, rope, identification tag, weight) was noted.

Determination of percentage cover of tunicate species

The percentage cover of the 5 species of tunicates on the bottom surface of each PVC plate was determined by visual examination. Categories for the percentage cover were 0 (absent); 1: < 25% coverage (low); 2: 25–50% coverage (moderate); 3: 51–75% coverage (high) and 4: >75% coverage (very high). A value of "1" was assigned to collectors where one to a few tunicates were present on surfaces other than plates (see above).

The average % cover for each species of tunicate at each station was determined by calculating the average coverage category for the duplicate collectors in each of the three deployment periods, and then calculating the average of the three values. Variable coverages were noted among plates on the same collector; often 2 or 3 different coverage categories were noted on a single collector. Similar variability in coverage was also noted between duplicate collectors.

Community outreach and education

In an effort to promote community based monitoring, an Invasive Tunicates Brochure and waterproof identification cards were produced and circulated to members of the general public, processing plant managers, aquaculture growers, fish harvesters, and boaters. An Invasive Species Monitoring Booklet produced in 2009 by DFO, Science – Quebec Region was also circulated. A Tunicate Identification Poster was produced and mounted in a prominent location at each station and replaced when necessary. A toll free telephone number and e-mail address to report sightings of tunicates were featured on all publications. Verified tunicate reports were recorded as anecdotal observations. All records of non-indigenous tunicates and other aquatic invasive species listed above were added to the DFO-Science National AIS Database.

Results

General monitoring: 2006–2009

The pattern of occurrence of tunicates was similar in all years (Table 1); *B. schlosseri* was the dominant species of tunicate reported (69 – 84% of records), followed by *C. intestinalis* (39

– 53% of records) and *B. violaceus* (19 – 50% of records). The number of stations where *C. intestinalis* was observed increased from 2006 to 2007 and onward (39% to 53%). The distribution of *B. violaceus* increased from 19% of stations in 2006 to 50% of stations in 2009. The number of stations without tunicates declined between 2006-2008 (23–29% of records) and 2009 (15% of records). *S. clava* and *D. vexillum* were not observed in Nova Scotia during the course of this study.

There was a great deal of variability in tunicate coverage (often two or three separate categories) between collector plates on the same collector, between duplicate collectors deployed during the same monitoring period, and among the three deployment periods at a given site.

In 2006, *C. intestinalis* was present with low coverages at Port Bickerton East (Stn 30) and Whitehead Basin (Stn 36) on the east coast, and in Sydney (Stn 62) (Appendix 1). Low to moderate coverages were recorded from Digby (Stn 1) to Gunning Cove (Stn 11), from Lunenburg (Stn 18) to Chester (Stn 21), in Halifax Harbour (Stn 24) and on Isle Madame (Stns 42 and 43) (Figures 1 and 2) (Appendix 1). Coverage was high in Chester. *B. schlosseri* was widely distributed with generally low coverages, but moderate coverages were noted at Gunning Cove (Appendix 1). Low coverages of *B. violaceus* were noted in the Lobster Bay area (Stns 6 and 7), at Port La Tour (Stn 9), in the Corkum's Island (Stn 17), and Lunenburg areas, on Isle Madame, and at Big Bras D'Or (Stn 76), and Cheticamp (Stn 71) in Cape Breton (Appendix 1). All three species of tunicates were present at Wedgeport (Stn 6), Camp Cove (Stn 7), Ingomar (Stn 10), Corkum's Island, and Lunenburg, and in Arichat (Stn 42) on Isle Madame. The north and most of the east coasts were free of tunicates (Figure 1).

In 2007, *C. intestinalis* was found along the west and south coasts, in Halifax Harbour (Stn 24), in the Whitehead (Stn 36) to Canso (Stn 38) area and in St. Peter's (Stn 47), with a new record in North Sydney (Stns 63) (Figures 1 and 2). Coverages on collector plates were generally higher along the west and south coasts in 2007 compared with 2006 (Appendix 1). Heavy coverages were noted at Camp Cove, Clark's Harbour, Ingomar, Shelburne, and Lockeport (Stns 7, 8, 10, 12 and 13, respectively), while coverage was very heavy at Lunenburg (Stn 18), Indian Point (Stn 19), and Chester (Stn 21) (Appendix 1). The "patchy" nature of tunicate coverage within a high coverage area is exemplified by *C. intestinalis:* low coverages were noted at Meteghan (Stn 2) and Port La Tour (Stn 9), while coverage at Gunning Cove (Stn 11) was moderate and the species was absent from stations in the Yarmouth area (Appendix 1). Coverage in Cape Breton and on the east coast was low (Appendix 1). *B. schlosseri* was widely distributed in 2007 with generally low coverages (Appendix 1). Moderate coverages were noted at Wedgeport (Stn 6), St. Peter's and Cheticamp (Stn 71), while coverage was heavy at Cape Canso (Stn 39) (Appendix 1). *B. violaceaus* was found on the southwest and south coast, in the Lunenburg to Chester area, at Cape Canso, North Sydney, and Cheticamp (Stn 71). Stations with these 3 tunicate species present were usually located on the west and south coast, with the exception of Cape Canso and North Sydney. As in 2005 and 2006, no tunicates were found on the north coast.

In 2008, *C. intestinalis* was again present from Digby (Stn 1) to Chester (Stn 21), at Whitehead (Stn 36), on Isle Madame (Stns 44 and 45) and at St. Peter's (Stn 47) (Figures 1 and 2). New records were noted for Inverness (Stn 72), Dingwall (Stn 69), and Gabarus (Stn 57) in Cape Breton. Coverage was high overall on the southwest to south coasts, with very high levels of coverage at Ingomar, Gunning Cove (Stn 11), Lunenburg (Stn 18), and at Indian Point (Stn 19) (Appendix 1). Coverage for this species was "patchy", as was observed in 2007: low in Digby and Chester (Stn 21) and moderate at Meteghan (Stn 2) and Shelburne (Stn 12). Coverage of *C. intestinalis* was very high at Petit de Grat (Stn 44) on Isle Madame, but low at all other locations in Cape Breton (Appendix 1). *B. schlosseri* was widely distributed in 2008, with many new reports in Cape Breton (Appendix 1). Heavier infestations, compared with 2006 and 2007, were observed in Wedgeport (Stn 6), Moose Harbour (Stn 15), Indian Point, and Chester on the south coast, and at Eddy Point (Stn 40), and in Little Harbour (Stn 46) and Cheticamp (Stn 71) on the Cape Breton coast. Collectors deployed at Whycocomagh, Gillis Cove, and Morrison's Cove (Stns 51, 48 and 49, respectively) in the Bras D'Or lakes had very high coverages, while Portage (Stn 50) collectors had high coverages (Appendix 1). Low coverages of *B. violaceaus* were found at many of the same locations as in 2007, with new records at Yarmouth Bar (Stn 4), St. Peter's, and Little Harbour (Appendix 1). Stations with

Table 1. Number of stations monitored, anecdotal reports, and presence and absence of tunicates in Nova Scotia, Canada, in 2006 through 2009. *Styela clava* and *Didenmum vexillum* were not recorded as present in any survey year. * indicates the inclusion of an anecdotal report in the percentage presence.

Year	Number of Stations	Number of Anecdotal Reports	*Ciona intestinalis* presence	*Botryllus schlosseri* presence	*Botrylloides violaceus* presence	Number of stations without tunicates
2006	61	1	39%	72%	19%*	23%
2007	35	1	50%*	69%*	37%	29%
2008	55	4	47%*	71%*	38%	27 %
2009	62	1	53%	84%*	50%*	15%

Table 2. Ranges of water temperature, salinity, and dissolved oxygen at general monitoring stations in Nova Scotia, Canada, where *Ciona intestinalis*, *Botryllus. schlosseri*, and *Botrylloides violaceus* were present in years 2006 through 2009. Values given are the minimum and maximum point values recorded at deployment or retrieval of monitoring collectors, with the exception of the salinity values indicated. Minimum water temperatures were taken during collector deployment in May.

Species	Year	Temperature (°C)	Salinity	Oxygen(% sat.)	Oxygen (mgL^{-1})
C. intestinalis	2006	8.3 – 20.9	22.4 – 33.1	54.2 – 88.4	4.77 – 8.25
	2007	5.9 – 21.1	~22.0 – 32.7[a]	67.2 –120.6	3.93 – 10.78
	2008	7.2 – 20.9	19.4 – 33.2	32.5 – 97.0	2.97 – 10.44
	2009	4.8 – 22.0	~26.5– 31.8[b]	57.2 –124.8	4.28 – 11.87
B. schlosseri	2006	8.0 – 26.9	13.0 – 33.1	43.0 – 96.4	3.93 – 8.72
	2007	7.3 – 23.4	~16.0 –32.7[c]	69.6 –120.6	3.37 – 10.78
	2008	7.2 – 20.9	17.8 – 33.2	32.5 – 98.0	2.97 – 10.44
	2009	5.2 – 24.3	~17.6 – 31.8[d]	57.9 – 124.8	4.28 – 13.75
B. violaceus	2006	8.6 – 20.9	26.7 – 33.0	43.0 – 96.4	3.93 – 8.72
	2007	7.5 – 20.9	~28.0 –32.7[e]	43.0 – 116.0	3.93 – 9.95
	2008	8.2 – 20.9	19.4 – 33.2	33.0 – 97.0	2.98 – 10.44
	2009	5.2 – 22.0	23.5– 31.8	63.5 – 114.9	4.28 – 11.87

[a]Lowest salinity is average of Stn 47 (St. Peter’s) measurements
[b]Lowest salinity is average of Stn 15 (Moose Harbour) measurements
[c]Lowest salinity is average of Stns 52 (Orangedale) and 53 (Nyanza) measurements
[d]Lowest salinity is average of Stns 51 (Whycocomagh) and 53 (Nyanza) measurements
[e]Lowest salinity is average of Stns 6 (Wedgeport) and 71 (Cheticamp) measurements

3 tunicate species present were concentrated on the west and south coasts, as in 2006 and 2007, and in the Isle Madame area, along with several new stations in Cape Breton: Gabarus (Stn 57), Alder Point (Stn 64), Dingwall (Stn 69), and Inverness (Stn 72). Stations monitored on the north and east coasts were free of tunicates.

The number of stations with infestations of *C. intestinalis*, *B. schlosseri*, and *B. violaceus* was highest in 2009 (Figures 1 and 2). *C. intestinalis* was found at most stations on the west and south coasts, and in the Isle Madame area. Its presence was confirmed at Havre Boucher (Stn 79), following an anecdotal report in 2008. Coverages were moderate to high at many sites on the west and south coasts, with several moderately infested sites (North Sydney, Eddy Point and Little Harbour) in Cape Breton and high coverage at Petit de Grat (Stn 44) (Appendix 1). *B. schlosseri* was widespread in 2009, with generally low coverages, and was noted at several new stations: Dundee Marina (Stn 87) and East Bay (Stn 85) in the Bras D’Or lakes, and at Ingonish (Stn 67) and Main a Dieu (Stn 59) on the Cape Breton coast. The highest coverages were noted in the Chester-Hubbards area (categories 3 and 2, respectively) and many locations in Cape Breton (Stn 56, Fourchu; Stn 57, Gabarus; Stn 62, Sydney; Stn 64, Alder Point; Stn 67, Ingonish; Stn 69, Dingwall; Stn 70, Bay St. Lawrence and Stn 72, Inverness) had higher infestations than were observed in 2008 (Appendix 1). *B. violaceaus* was again present at many stations, with low infestations on the south coast, Isle Madame, and coastal Cape Breton (Appendix 1). This species was recorded for the first time at Port Mouton (Stn 82), Fourchu, Ingonish, and Main a Dieu. With the exception of Havre Boucher, the north coast of Nova Scotia remained free of tunicates. Monitoring stations on the east coast were not maintained in 2009, so information from this region is absent.

Targeted monitoring in 2006

In the Halifax - St. Margaret's Bay area (Figure 1), *C. intestinalis* was found at all sites in Halifax Harbour, with moderate coverage at the Armdale Yacht Club (Stn 401), and a heavy infestation at the Bedford Basin Yacht Club (Stn 403), but this species was not found in St. Margaret's Bay (Stns 22, 23, 404, 405 and 406). *B. schlosseri* was found at 7 of 9 stations in the area, with low coverage, and was absent in French Village harbour (stn 406) and at the Royal Nova Scotia Yacht Squadron (Stn 402) (Figure 1). *B. violaceus* was present only in French Village harbour. There were no stations where all 3 species of tunicates were present.

In the Sandy Point - Port La Tour area (Figure 1), *C. intestinalis* was found at 5 of 8 stations, with heaviest coverage at Gunning Cove (Stn 11). *B. schlosseri* was found at 7 of 8 stations, with moderate coverage at Gunning Cove and Upper Port La Tour (Stn 411). *B. violaceus* was found at Ingomar (Stn 10), Blanche (Stn 410), and Upper Port La Tour. Smithsville (Stn 409) was free of tunicates. Three stations, Ingomar, Blanche, and Upper Port la Tour, had all 3 species of tunicates present.

In the Canso - Isle Madame area (Figure 2), Arichat (Stn 42) was the only station where all 3 species of tunicates were present. *C. intestinalis* was present at an additional 2 stations: Queensport (Stn 412) and Eddy Point (Stn 40), while *B. schlosseri* was present at 8 of 9 stations, with the heaviest coverage observed at Eddy Point (moderate) and River Bourgeois (Stn 417, moderate). Cooks Cove (Stn 413) was the only station free of tunicates; this was a very small station limited to small recreational boat traffic.

No clear pattern of distribution was observed for *B. schlosseri* or *B. violaceus* in any of the targeted areas. *C. intestinalis* was concentrated in Halifax Harbour in Area 1, a location with international shipping and three yacht clubs with US and local recreational traffic. The intensity of coverage varied from station to station in each area; stations with no tunicate presence were often close to stations with high coverage. The type, or size, of a station did not seem to be related to tunicate coverage. In Area 2, for example, the highest coverages were found in a small cove with a processing plant (Stn 410) and at 2 small ports (Stns 411 and 10), while fewer tunicates were found at two other small ports (Stns 9 and 11) (Appendix 1). As was evident from the general monitoring results in all years, tunicate distributions are "patchy", with spatial differences in the intensity of infestation and species present within a targeted area.

Environmental ranges for presence of C. intestinalis, B. schlosseri and B. violaceus

C. intestinalis and *B. violaceus* were present at stations with water temperatures up to 22°C, while *B. schlosseri* was found at several stations in the Bras D'Or lakes with higher summer temperatures in the range of 24 – 26°C (Table 2). Examination of hourly temperature records gathered in 2008 and 2009 confirmed that sustained summer temperatures in the 20 – 24°C range were encountered at several stations on the south coast, in the Bras D'Or lakes, and at St. Peter's.

Minimum salinity values tolerated by the three species of tunicates were difficult to determine in some cases, as heavy spring and fall rains and surface runoff events were observed at several stations. This phenomenon was particularly evident at Shelburne in 2007, where *C. intestinalis*, *B. schlosseri,* and *B. violaceaus* were exposed to salinities of 6.7, and again in 2009, where *C. intestinalis* and *B. schlosseri* were exposed to salinities of 10.9 (Table 2). The 2007 and 2009 salinity data were examined more closely to identify "low salinity stations", mostly in the Bras D'Or lakes, and average seasonal salinity values were calculated to achieve more appropriate salinity values. Of the 3 species, *B. schlosseri* survives in the lowest salinities (13 – 17.8), followed by *C. intestinalis* (19.4 – 26.5), and *B. violaceus* (19.4 – 28.0).

All three species of tunicates were found in waters of low (32.5%; 2.97 $mg.L^{-1}$) to high (116 – 124.8%; 8.75 – 10.78 $mg.L^{-1}$) dissolved oxygen concentrations (Table 2).

Public Outreach and Education

Although no formal measures were made to determine the success of our public outreach and educational initiatives, we estimate that several hundred people were engaged annually through personal contact, follow-ups on reports made to the reporting telephone line and e-mail, and at public and industry meetings and events. Indeed, with each passing year, there seemed to be more interaction with the general public at monitoring stations and an increasing number of requests for information, identification materials, and instructions on proper cleaning of boats to remove tunicates.

Discussion

Spatial and year-to-year patterns in tunicate distribution

The distribution and intensity of tunicate coverage at monitoring stations throughout Nova Scotia can best be described as discontinuous or "patchy" in space and "sporadic" in time. The presence of non-indigenous tunicates in an area is the result of (1) new introductions from native waters, (2) spread or range expansion, and (3) stepping-stone type introductions from one local port or harbour to another, usually attached to boat hulls or fishing gear (Clarke and Therriault 2007). During the course of this study we documented established, continuous populations of *C. intestinalis* in the Lobster Bay area, on the south coast, in the Halifax area, and on Isle Madame, and populations of *B. schlosseri* in many areas including the Bras D'Or lakes. Stations with a continuous tunicate presence may reflect sites of initial, older introductions, with range expansion on a small scale (i.e., within a bay). New introductions from native waters, or port-to-port spread from established populations may account for wider, or discontinuous distributions.

Natural dispersal is generally limited in ascidians and restricted to gametes and larval stages (Lambert and Lambert 1998; Lambert 2005). This phenomenon may explain the high variability in coverage noted among plates of duplicate collectors deployed at the same station. Information gained from targeted monitoring in 2006 also suggests that natural dispersal may account for the "pockets" or patches of tunicates observed among closely spaced stations. Indeed, Osman and Whitlatch (1995) indicate that most ascidian larval settlement occurs within 10 m of the adults. Svane and Havenhand (1993) have shown that larval dispersal of *C. intestinalis* in native Swedish waters is restricted inside a bay. Moreover, dense aggregations of both adults and newly settled *C. intestinalis* may result as many developing larvae are retained in adhesive mucus strings (Svane and Havenhand 1993).

The expanding distributions documented at general monitoring stations during the course of this study are thus most likely stepping-stone introductions, as adult tunicates "hitchhike" on boat hulls to new locations where they successfully spawn. Many ports and marinas selected for monitoring had moderate to high volumes of commercial fishing and recreational traffic, which probably resulted in numerous, port-to-port introductions in mainland NS and in Cape Breton. Darbyson et al. (2009) concluded that, nearby in the southern Gulf of St. Lawrence, recreational boaters were more likely vectors for new tunicate introductions than commercial fishermen. Many colonial species, such as *B. schlosseri* (Worcester 1994) and *D. vexillum* (Lengyel et al. 2009), can also spread to new locations as floating fragments, or by rafting on eelgrass or algae. The arrival of *C. intestinalis* on aquaculture sites in the Whitehead area may also be the result of an introduction through the vector of contaminated shellfish product or gear from an infested area (McKindsey et al. 2007).

In terms of new locations reported in this study, *C. intestinalis* is present and persistent in Digby, present along the east coast from Country Harbour to Whitehead and Canso, and present in many locations along Chedabucto Bay. Several infested sites are now documented in coastal Cape Breton, including Dingwall Harbour in northern Cape Breton. The shores of the Bras D'Or lakes appear to be free of *C. intestinalis* to date, although its presence in St. Peter's at the southern entrance to the Lakes may reflect new introduction(s) on recreational boat hulls through the St. Peter's Canal from Isle Madame or beyond. Its presence in North Sydney is troubling, as this high traffic port is linked to the south coast of Newfoundland, where *C. intestinalis* has not yet been recorded but where *B. schlosseri* and *B. violaceus* are now present (C. McKenzie, pers. comm.). Its presence in Sydney is also of concern, as there is a large volume of recreational boat traffic from the area into the Bras D'Or Lakes, and to coastal Cape Breton. Given the range of salinities observed in the Bras D'Or Lakes (13–21), however, this may not be an optimal habitat for the establishment of large populations of *C. intestinalis*.

B. schlosseri has been reported as present in Nova Scotia for several decades and anecdotal reports have indicated that it has been widespread in the Bras D'Or lakes for several years. Indeed, *B. schlosseri* is widely distributed in all areas of the province, with the exception of the north coast. Unlike infestations of *C. intestinalis*, which may have negative impacts on shellfish growth and survival on aquaculture sites, *B. schlosseri* is generally regarded as merely a nuisance species which must be removed from gear and shellfish during processing. We have also observed several instances of extensive fouling on wharves, floating docks, buoys, and monitoring collectors since 2007 (i.e., Whycocomagh, Gillis Cove, Portage and Morrison's Cove in 2008; St. Peter's in 2007, 2008 and 2010; Wedgeport in 2007 and 2008; Hubbards and Chester in 2008 and 2009).

Of the three tunicate species encountered during this study, *B. violaceus* has shown the greatest expansion in the number of locations affected. The number of stations with *B. violaceus* present more than doubled from 2006 (11 of 62 stations) to 2009 (29 of 64 stations). The species is now well established on the southwest and west coasts, and at many sites on coastal Cape Breton, which is most likely evidence of new introductions from mainland Nova Scotia, or from the Isle Madame area.

The north coast of Nova Scotia appears to be the only area that is tunicate free, although *C. intestinalis* was recorded in Havre Boucher in 2008 and 2009. This area is at considerable risk for tunicate invasions from the southern Gulf of St. Lawrence and the south coast of PEI, given the volume of recreational and commercial traffic (Darbyson et al. 2009). While *C. intestinalis* and *B. schlosseri* are now well established in the Whitehead area at the tip of the east coast, anecdotal evidence from one shellfish aquaculture site at Ship Harbour indicates that this area is still free of tunicates (K. Lind, pers. comm.). Little monitoring has been done along the east coast of NS since 2006, representing a significant knowledge gap. It is crucial that monitoring, education and outreach efforts continue in these areas to help prevent new introductions of non-indigenous tunicates.

The intensity of coverage showed variability from year to year during the course of this study. Similar variability has also been shown in recruitment studies for *C. intestinalis* (Carver et al. 2003; Howes et al. 2007; Vercaemer et al. 2011). While our observations on the intensity of coverage on collectors at monitoring stations are somewhat discontinuous because different stations were monitored in different years, year-to-year patchiness can be inferred over broader areas by examining data from stations monitored in 3 or 4 consecutive years (Appendix 1). In Digby, for example, low coverages of *C. intestinalis* were evident in 2006, 2008, and 2009, but the species was absent on the collectors in 2007. In Wedgeport, where three species were present in all years, *C. intestinalis* coverage was moderate in 2009, but light in other years, while *B. schlosseri* coverage was moderate in 2007 and 2008, the same years when populations of *B. violaceus* were also moderate in intensity. At Hubbards, tunicate infestations gradually grew with time; *B. schlosseri* was the only species present in 2006 and 2008, but by 2009, its coverage had increased to moderate, and low infestations of *C. intestinalis* and *B. violaceus* were also evident. While the "wax and wane" of species was evident at many stations over the course of this study, there was a general trend toward heavier infestations, and for an increase in the number of species present at many stations by 2009 (Appendix 1). Even species that were not detected for a year or two generally re-occurred with similar or increasing coverages on monitoring collectors.

Environmental tolerance of tunicates in Nova Scotia

Environmental variables such as temperature, salinity, light, and hydrodynamics are important in determining the distribution of ascidians (Lambert 2005; Lambert and Lambert 2003; Carver et al. 2006a, b). The range of temperature and salinities associated with records of *C. intestinalis*, *B. schlosseri*, and *B. violaceus* noted in this study are in general agreement with previously documented ranges.

C. intestinalis tolerates a wide range of temperature and salinity (Carver et al. 2006a). While Scandinavian populations rarely experience temperatures below 1°C, in Atlantic Canada the species survives in waters from -1 to 22°C, and in a few protected bays with temperatures up to 27°C. Its ability to survive harsh winters may be due to its occupation of refugia at depth for overwintering (Vercaemer et al. 2011). We observed healthy populations in areas with summer temperatures in the 22°C range, and we noted healthy, overwintered adults in the spring of 2009 and 2010 attached to floating docks and lines at many locations on the southwest and south coasts. While *C. intestinalis* is euryhaline, with tolerances between 12 and 40, most populations are found in salinities above 30 (Carver et al. 2006a). *C. intestinalis* was present at monitoring stations with salinities as low as 19.4, and was present annually in one location, Shelburne, where spring runoff and fall and spring rains resulted in salinities below 10 for periods of time. In southern California, Lambert and Lambert (1998) noted that winter rains caused massive die-offs of ascidians, which were followed by large recolonizations in the spring.

As with temperature, the tolerated salinity ranges depend on ecotype (Carver et al. 2006a). Sustained unfavourable temperature and salinity conditions (high temperature – low salinities) have been shown to be detrimental to both survival and growth of young *C. intestinalis* in a challenge experiment (Vercaemer et al. 2011).

Botryllids are generally considered warm water species (Carver et al. 2006b). *B. schlosseri* tolerated higher summer temperatures and lower salinities, compared with *C. intestinalis*, while *B. violaceus* was found in a range of environmental conditions similar to *C. intestinalis*. *B. schlosseri* was the dominant tunicate in the Bras D'Or lakes, where summer temperature can rise to 25°C, and salinity can be as low as 13. This observation is in contrast with studies that suggest that the species is limited by salinities below 18, although older colonies tolerate a wider salinity range than younger ones (Brunetti et al. 1980). Epelbaum et al. (2009) conducted experimental challenge studies and found that *B. schlosseri* is slightly more euryhaline than *B. violaceus*. Higher temperatures favour growth of *B. violaceus* over *B. schlosseri*, as has also been observed by McCarthy et al. (2007). *B. schlosseri* survived in water temperatures of 10 to 25°C and at salinities of 14 to 38, with growth occurring between 20 and 38. *B. violaceus* survived in waters with temperatures of 5 to 25°C and at salinities of 20 to 38, but growth on hard substrata was only noted in water temperatures of 15 to 25°C and at salinities between 26 and 38 (Epelbaum et al. 2009). While these conditions for growth are well within the range of environmental conditions recorded during monitoring in Nova Scotia, *B. violaceus* has not yet been recorded in the inner Bras D'Or lakes, where salinities commonly range from 13 to 25.

Absence of Styela clava and Didemnum vexillum

We did not detect *S. clava* in Nova Scotia at any station during this study, or during subsequent work conducted in 2010. Populations of *S. clava* have declined somewhat in recent years in PEI (Ramsay et al. 2008), but given the impact that this species has had on shellfish aquaculture in that province (Clarke and Therriault 2007), it is crucial to prevent its introduction into Nova Scotian waters. Stations were monitored in the southern Gulf of St. Lawrence, close to PEI, where vessel traffic is common between the two provinces, and a Rapid Assessment was conducted at Caribou, NS, in August 2010, to determine if tunicates were present at the NS-PEI Ferry terminal and in Caribou Harbour. The concerted efforts to contain this species in PEI described by Locke et al. (2009) may have succeeded to date. The DFO Introductions and Transfers Committee, which works with the Nova Scotia Department of Fisheries and Aquaculture and the shellfish aquaculture industry, conducts reviews of requests to transfer shellfish spat and brood-stock from other provinces into Nova Scotia, and between sites within the province. This process ensures that introductions are not made from tunicate infested areas into non-infested waters. Combined with industry awareness of the risks posed by new tunicate introductions to aquaculture sites, it is possible that *S. clava* may not arrive in Nova Scotia through an inadvertent industry transfer. However, the risk of "hitchhiking" on drifting material or on boat hulls and equipment is still very high as recreational and commercial fishing boats make regular trips between PEI and NS (Locke et al. 2009). *S. clava* tolerates shallow (< 25m) environments with temperatures of 2 to 23°C and salinities of 20 to 30 (Clarke and Therriault 2007), and while some areas of the province may be unsuitable, conditions on the Fundy, west, and south coasts of NS, and most of coastal Cape Breton may be suitable, provided that winter water temperatures are not severe.

D. vexillum, present on the American side of Georges Bank and along the Gulf of Maine coast as far north as Eastport, Maine, has not been recorded in the Bay of Fundy (LeGresley et al. 2008; Martin et al. 2010), or at any stations monitored in Nova Scotia between 2006 and 2010. This species has the potential for distribution from Georges Bank with fishing gear, or as dislodged fragments carried by currents (Lengyel et al. 2009). Fragments of *D. vexillum* can reattach to hard substrata and thrive in new locations (Bullard et al. 2007b). The west and south coasts of Nova Scotia are in direct line with ocean currents originating from the region where the species is presently distributed and there are numerous home ports to Georges Bank scallop vessels on these coasts, so careful monitoring in this area is necessary. *D. vexillum* grows on hard substrates and surfaces, in water temperatures ranging from -2 to 24°C (Lambert 2005), and salinities above 25 (Daniel and Therriault 2007), and there are many suitable environments for its establishment in this area. The species is an aggressive colonizer, with the ability to overgrow and alter native plant, fish, and invertebrate communities (Daniel and Therriault 2007), so it is crucial to detect new populations at an early stage to prevent further spread.

In conclusion, given that the ranges of environmental conditions present along the coastline of Nova Scotia are suitable for the establishment of many non-indigenous tunicate species (Locke 2009), and that many important transport vectors for their introduction and spread are active in Nova Scotian waters, it is imperative that monitoring efforts that report tunicate distributions, arrival, and spread are continued. Education and outreach to shellfish growers, the fishing industry and recreational boaters are essential in order to prevent the human-mediated spread of tunicates to new locations. Research to better understand environmental constraints on tunicate growth and survivorship may facilitate better management practices on shellfish aquaculture sites. In concert with the development of new mitigation strategies and techniques, the impact of non-indigenous tunicates on coastal habitats and the shellfish aquaculture industry may be reduced.

Acknowledgements

Funding for this monitoring project was provided by Fisheries and Oceans Canada, Aquatic Invasive Species Program. We are grateful to the following individuals and organizations who monitored stations and provided data: Lorne Penny, DFO Maritimes Region; Renée Bernier, Carla Hicks, Erica Watson and Andrea Locke, DFO Gulf Region; Anaïs Lacoursiere-Roussel, DFO Quebec Region; Andrew Bagnall, Nova Scotia Department of Fisheries and Aquaculture; Katherine Jones and Project Unidentified Foreign Organisms (PUFO), Cape Breton University; Shelly Denny, Unama'ki Institute of Natural Resources; Allison MacIsaac and John Johnson, Eskasoni Fisheries and Wildlife Commission, and Andy Sharpe and Marika Godwin, Clean Annapolis River Project (CARP). Technical and in-kind support were also provided by the Nova Scotia Aquaculture Association; Carl McDonald and Jeff Graves, Fishermen and Scientists Research Society; Alida Bundy, Nell denHeyer, Peter Comeau, Jim Simon and the late Stephen Nolan, Population Ecology Division, DFO Maritimes Region, and shellfish growers Peter Darnell, Bruce Hancock and Robin Stuart. We express our appreciation to numerous Harbour Authorities, Yacht Club and Marina Managers and Fish Plant Managers throughout Nova Scotia who allowed us access to their facilities throughout the course of this project. Special thanks are extended to Jordan Ouellette-Plante, for many hours of data verification and preparation of the tables and figures. Thanks are also extended to the reviewers for their useful comments which improved the manuscript.

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Supplementary material

The following supplementary material is available for this article.

Appendix 1. Location of invasive tunicates at monitoring stations in Nova Scotia, Canada in 2006 through 2009.

This material is available as part of online article from:
http://www.aquaticinvasions.net/2011/AI_2011_6_4_Sephton_etal_Supplement.pdf